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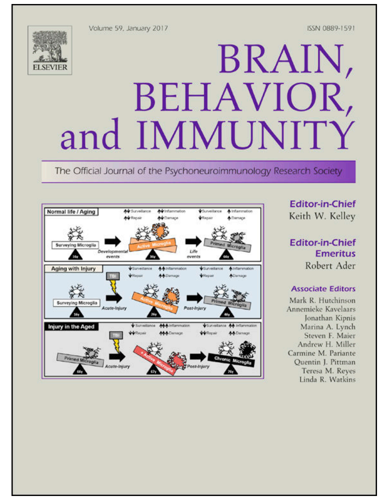

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Factors predisposing to humoral autoimmunity against brain-antigens in health and disease:

Analysis of 49 autoantibodies in over 7000 subjects

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ABSTRACT

Background: Circulating autoantibodies (AB) against brain-antigens, often deemed pathological, receive increasing attention. We assessed predispositions and seroprevalence/characteristics of 49 AB in >7000 individuals.

Methods: Exploratory cross-sectional cohort study, investigating deeply phenotyped neuropsychiatric patients and healthy individuals of GRAS Data Collection for presence/characteristics of 49 brain-directed serum-AB. Predispositions were evaluated through GWAS of NMDAR1-AB carriers, analyses of immune check-point genotypes, *APOE4* status, neurotrauma. Chi-square, Fisher's exact tests and logistic regression analyses were used.

Results: Study of N=7025 subjects (55.8% male; 41±16 years) revealed N=1133 (16.13%) carriers of any AB against 49 defined brain-antigens. Overall, age dependence of seroprevalence (OR=1.018/year; 95% CI [1.015-1.022]) emerged, but no disease association, neither general nor with neuropsychiatric subgroups. Males had higher AB seroprevalence (OR=1.303; 95% CI [1.144-1.486]). Immunoglobulin class (N for IgM:462; IgA:487; IgG:477) and titers were similar. Abundant were NMDAR1-AB (7.7%). Low seroprevalence (1.25%-0.02%) was seen for most AB (e.g. amphiphysin, KCNA2, ARHGAP26, GFAP, CASPR2, MOG, Homer-3, KCNA1, GLRA1b, GAD65). Non-detectable were others. GWAS of NMDAR1-AB carriers revealed three genome-wide significant SNPs, two intergenic, one in *TENM3*, previously autoimmune disease-associated. Targeted analysis of immune check-point genotypes (*CTLA4, PD1, PD-L1*) uncovered effects on humoral anti-brain autoimmunity (OR=1.55; 95% CI [1.058-2.271]) and disease likelihood (OR=1.43; 95% CI [1.032-1.985]). *APOE4* carriers (~19%) had lower seropositivity (OR=0.766; 95% CI [0.625-0.933]). Neurotrauma predisposed to NMDAR1-AB seroprevalence (IgM: OR=1.599; 95% CI [1.022-2.468]).

Conclusions: Humoral autoimmunity against brain-antigens, frequent across health and disease, is predicted by age, gender, genetic predisposition, and brain injury. Seroprevalence, immunoglobulin class, or titers do not predict disease.

INTRODUCTION

Autoantibodies (AB) in general and brain-directed AB in particular have received increasing attention in the last decades (1-6). Human protein microarrays containing large numbers of antigens in a native conformation, identified abundant and ubiquitous natural IgG AB in human sera, many of which apparently belong to the normal autoimmune repertoire (7-9). Brain-directed AB, dependent on the antigen, can substantially modulate brain function - provided they gain sufficient access to the brain (10, 11). This access is usually limited, but quite excessively possible upon intrathecal synthesis or disruption of the blood-brain-barrier (BBB), as found e.g. due to genetic predisposition (Apolipoprotein E ε4 allele [*APOE4*] genotype), after traumatic brain injury or stroke, during systemic inflammatory processes and encephalitides, or even under anesthesia (12-16). The *'how-when-where'* conditions of intrathecal AB synthesis are still obscure. AB against the N-methyl-D-aspartate-receptor subunit-NR1 (NMDAR1-AB), exhibiting the highest seroprevalence presently known for anti-brain AB (10, 17), are particularly interesting. By acutely downregulating NMDAR1 surface expression, they can exert a spectrum of behavioral, neurological, or psychopathological effects, resembling the pharmacology of ketamine-like agents, even including antidepressive properties (3, 9, 18, 19). In case of sudden BBB leakiness, they bind in large amounts to brain tissue which virtually acts as 'targeting sponge' or 'immunoprecipitator' of these AB, massively and specifically extracting them from the circulation (11). Similarly, other brain-directed AB, e.g., against CASPR2 or GABA-a, can bind to respective sites in brain and exert their specific effects (4, 20-23). Intriguingly, Lupus AB can act as positive allosteric modulators at GluN2A-containing NMDAR and impair spatial memory (24). Therefore, serological testing for brain-directed AB is an important diagnostic measure in patient care but any expected contribution to clinical syndromes has to be carefully appraised in each individual context.

Associations of e.g. NMDAR1-AB with teratoma/tumors (18), or with infections like Herpes (25) or Influenza A/B (10, 11) were reported. However, the (patho)physiological roles of AB – e.g., those of NMDAR1-AB, highly seroprevalent across mammals (26) - are still incomprehensible. Notably, all naturally occurring NMDAR1-AB have pathogenic potential, irrespective of epitope and immunoglobulin (Ig) class (27). Overall, considerable mysteries have remained for both scientists and clinicians regarding syndromic or disease relevance of AB. This lack of understanding is not infrequently of disadvantage for AB-carrying patients, who may get immunosuppressive treatment too hastily (28). We have to ask under which conditions brain-directed AB in serum do gain pathological significance. Can they help distinguish between health and disease states? In other words, are carriers of these AB more likely to belong to disease groups? Are particular Ig classes more relevant than others regarding disease indication? In a nutshell, do peripheral serum titers by themselves tell us anything meaningful for diagnostic conclusions? Can we identify any solid predictors of seroprevalence?

Considering the high and ever-increasing clinical relevance of these questions, the present study was designed to investigate seroprevalence and potential predictors of 49 brain-directed AB in >7000 subjects, healthy or suffering from neuropsychiatric diseases, i.e. schizophrenia, schizoaffective, affective, personality disorders, addiction, autism spectrum, stroke, neurodegenerative and autoimmune diseases. Importantly, we find age, gender, genetic predisposition and brain injury associated with serum AB. However, neither seroprevalence nor Ig class nor titers are solid predictors of any disease. These results question a straightforward pathogenic role of these brain-directed AB and should appeal for more fundamental research to gain a better understanding of their (patho)physiological significance.

METHODS AND MATERIALS

Subjects

Subject data collection in the scope of the extended GRAS (Göttingen Research Association for Schizophrenia) database has been approved by the ethical committee of the Georg-August-University of Göttingen (master committee) as well as by the respective local regulatories/ethical committees of collaborating centers, all in accordance with Helsinki Declaration (29). Individuals were recruited all across Germany and are mostly of German origin. A total of N=7,025 participants are evaluated in this study, with N=4,266 of them previously analyzed for 24 serum AB (17). Of the N=2,759 new individuals, N=1,030 are healthy controls (mostly anonymized blood donors of Department of Transfusion Medicine, Göttingen). The remaining 1,729 individuals add to existing subgroups of the GRAS Data Collection. The newly formed autoimmune group (n=701) and 37 individuals with neurodegenerative disorders were recruited at Department of Neurology, University Medical Center, Johannes Gutenberg University Mainz. For overview of antigens screened and AB seroprevalence in disease groups see Tables 1 and 2.

Serological analyses

Serological analyses were performed as described earlier (17). Briefly, serum samples of all individuals were tested for AB presence using biochip mosaics (Euroimmun, Lübeck, Germany) that contained nonfixed nitrogen-frozen tissue cryosections (4mm; rat hippocampus, monkey cerebellum) and recombinant cell substrates (formalin- or acetone-fixed transfected HEK293 cells). Recombinant protein (autoantigen) expression was validated by immunological methods employing human or commercially available monospecific animal antibodies. A total of 49 different neural antigens, previously associated with autoimmunity/autoimmune disease (2, 25, 30-54) were evaluated: NMDAR1, AGNA, AMPAR, amphiphysin, ANNA-3, AP3B2, AQP4, ARHGAP26, AT1A3, CARPVIII, CASPR2, CNTN1, CNTN2, CV2, DPPX, DRD2, ERC1, flotillin 1/2, GABA-a, GABA-b, GAD65, GFAP, GLRA1b, GluRD2, Homer-3, Hu, IgLON5, ITPR1, KCNA1, KCNA2, LGI1, Ma2, MAG, MBP, mGluR1, mGluR5, MOG, Myelin, neurexin, neurochondrin, NF155, NF186, PCA-2, recoverin, Ri, Sez6l2, Tr/DNER, Yo, Zic-4. Table 1 lists the most frequent antigens with respective references (2, 25, 30-54).

Genotyping

A semi-custom Axiom® myDesignTM genotyping array (Affymetrix, Santa Clara, CA, USA), based on a CEU (Caucasian residents of European ancestry from Utah, USA) marker backbone including 518,722 single nucleotide polymorphisms (SNPs), and a custom marker set including 102,537 SNPs was used for genotyping (detailed description (10)). A total of 493,925 variants passed quality control, had minor allele frequency > 0.05, were in Hardy–Weinberg equilibrium (p > 0.001) and therefore included in genetic analyses.

Genetic association analyses

PLINK v1.90 (55) was used for all genetic association analyses, including calculations of relatedness, principal components, and LD-based clumping (index variant *p* value threshold=0.01). A total of 254,250 variants and 5,393 individuals were available after these steps. For related individuals, one was randomly excluded in each pair (second to third-degree relatives, PIHAT > 0.185). We executed two different approaches: (ii) A genome-wide association study (GWAS) with NMDAR1-AB seropositivity as target phenotype (254,250 SNPs – Bonferroni threshold 1.97e-07) and (i) a hypothesis-driven strategy, including all 9 directly genotyped SNPs available in our array of immune-checkpoint genes (*cytotoxic T-lymphocyte-associated protein 4 [CTLA-4]*: rs231777, rs3087243, rs11571316; *programmed cell death protein 1 [PD-1]*: rs28680420; *programmed cell death protein ligand 1 [PD-L1]*: rs1411262, rs2890658, rs2297137, rs2297136, rs4143815; Bonferroni threshold 0.0055). All *p* values adjusted using "p.adjust" from R (56).

APOE Genotyping

APOE genotyping was done using KASP by Design assay (LGC/Biosearch™ Technologies, Berlin, Germany), targeting *APOE* SNPs rs7412 and rs429358, as described earlier (57). Plates were run using LightCycler® 480 II (Roche Diagnostics Ltd., Rotkreuz, Switzerland) and the measured values exported using LightCycler® 480 software (v1.5.0.39). Final genotype assignment was done using R (56).

Neurotrauma evaluation

Neurotrauma (NT) information was available for a subset of GRAS individuals (N=2061), all based on semi-standardized interviews that were additionally complemented by medical/discharge letters (Table 3). Based on conditions underlying categories, an **overall severity score** was calculated. For each individual, the NT with highest severity gave an initial rank. Any preceding or repeated NT, provoking potential accumulation of consequences, received additional NT scores, dependent on category, all summed up with initial rank (Table 3). Subjects were then dichotomously divided into 2 groups: Individuals with severity score of ≥2.5 considered as severe head injury (NT+) and <2.5 as not having had severe head injury (NT-).

Statistical Analysis

Chi-square tests and Fisher's exact tests were used to compare categorical variables between groups, with odds ratios and Wald confidence intervals calculated using R package "epitools" (58). Logistic regression analysis was employed to assess effects of disease status, age, gender, neurotrauma, *APOE4* genotype on seropositivity. Titer values were compared by Wilcoxon Rank Sum test with healthy controls as reference, using R package "e1071" (59). Statistical tests were conducted using R (56) with

RStudio (60). Statistical significance was set to 0.05 after Bonferroni correction where indicated. Figures were plotted using R package “ggplot2” (61) or GraphPad Prism (62).

RESULTS

Distribution of 15 most seroprevalent brain-directed AB across disease & health

A comprehensive overview of the 15 most seroprevalent of our selected 49 brain-directed AB across disease groups and health, i.e., NMDAR1-AB, amphiphysin, KCNA2, ARHGAP26, GFAP, CASPR2, MOG, Homer-3, KCNA1, GLRA1b, GAD65, Ma2, Yo, NF115, and AP3B2, is presented in Table 2. Information includes number of seropositive subjects, gender, mean age, Ig classes, and titer range. AB below cutoff of ≤0.25% were NF186, CNTN1, myelin, neurochondrin, flotillin1/2, CNTN2, IgLON5, AMPA, Sez6l2, recoverin, neurexin, mGluR1, ITPR1, GABA-b, LGI1, mGluR5, DRD2, CV2, Hu, Tr/DNER, AQP4, GluDR2, Zic-4, ERC1 *(data not shown)*. Non-detectable in N=7025 subjects were GABA-a, MBP, AT1A3, Ri, AGNA, CARPVIII, PCA-2, ANNA-3, DPPX, MAG, all expected to be extremely rare (63, 64). For brief biological description of the 15 most seroprevalent brain-directed AB see Table 1. Analysis of 49 defined anti-brain AB in serum of >7000 subjects, healthy or diagnosed with neuropsychiatric diseases, revealed >16% carriers of one or more of these selected AB.

Impact of disease status, age or gender on seroprevalence of brain-directed AB

Using logistic regression with any seroprevalence of all 49 brain-directed AB (no matter which Ig class or titer) as dependent variable, we first evaluated the overall impact of age, gender and disease versus health. Neither general disease status nor individual disease groups predicted AB seropositivity (all p>.05). The same negative result was obtained when only NMDAR1-AB, with highest seroprevalence, or all non-NMDAR1-AB together (all p>.05) were checked analogously. Age is strongly predictive of seropositivity when all 49 AB (OR=1.018/year, 95% CI [1.015-1.022], p=2.95e-21) or only NMDAR1-AB (OR=1.029/year, 95% CI [1.023-1.034], p=2.2e-27) are considered. However, age is just weakly predictive for non-NMDAR1-AB (OR=1.007/year, 95% CI [1.002-1.012], p=.0028) (Figure 1 A, B). In contrast, gender is not (yet) significantly associated with seropositivity when considering just NMDAR1-AB (p=.052). Here, significance is driven by the heterogeneous non-NMDAR1-AB 'bag' – with higher seroprevalence of males (OR=1.365, 95% CI [1.155-1.615], p=.0003) (Figure 1 A, B). Interestingly, but still unexplained, IgM seropositivity is not associated with gender in any of the tested scenarios (all p>.05).

Influence of anti-brain AB Ig class and titer regarding health or disease status

Evaluating the above obtained negative results on disease versus health status separately for the different Ig classes of brain-directed AB, including IgG, did not result in any appreciable association with disease (all p>.05). We then tested whether the distribution of AB titers was different between health and disease using Wilcoxon Rank Sum test. Positive individuals for either any disease or for each disease group separately were paired with matching healthy controls regarding age and gender to the maximum possible extent. For each individual, the highest titer (independent of Ig class) for each AB was selected, i.e., one individual could contribute to the comparison with 2 or 3 titers if seropositive for different AB. Importantly, no differences in titer distribution were found regardless of disease group (all p>.05).

Genome-wide association study of NMDAR1-AB carriers versus non-carriers

We next started a number of approaches to identify potential genetically predisposing factors for seroprevalence of AB against brain-antigens. An ideal readout for GWAS are NMDAR1-AB, most abundant for still unknown reasons, whereas low seroprevalence is seen for most other screened AB, some even being non-detectable. A Manhattan plot illustrating the GAS of NMDAR1-AB carriers is presented in Figure 1 C. GWAS revealed three genome-wide significant SNPs, two intergenic (rs155850 – risk allele T on chromosome3 and rs10159862 – risk allele G on chromosome10), and one intronic in *TENM3* (rs6820921 – risk allele C on chromosome4), a gene associated with childhood autoimmune diseases (65).

Targeted analysis of immune check-point genotypes (*CTLA4, PD1, PD-L1*)

Subsequently, we performed a hypothesis-driven analysis on 9 SNPs of immune-checkpoint genes, *CTLA-4*, *PD-1*, or its ligand, *PD-L1*. We had previously shown in a smaller sample that *CTLA4* genotypes predispose to serum NMDAR1-AB in humans (19). Now, we also included SNPs in *PD-1* and *PD-L1* in our analyses of 49 brain-directed AB in a population of N=5223 individuals with information available on all originally screened 9 SNPs, 5 of which were found here to be risk SNPs (*CTLA-4* - 2

SNPs, *PD-1* - 1 SNP, *PD-L1* - 2 SNPs; Table 4). A model, accumulating these 5 risk SNPs, and analyzing in a dichotomous fashion the presence of 0-3 versus 4-5 risk genotypes, uncovered effects not only on humoral anti-brain autoimmunity (OR=1.55; 95% CI [1.058-2.271]), but very interestingly also on disease likelihood (OR=1.43; 95% CI [1.032-1.985]). Subdivision of disease entities revealed that an association with 'classical' psychiatric diseases does not reach significance (p=0.223), whereas an association is observed between number of immune-checkpoint risk genotypes and probability of neurodegenerative disease (OR=2.04; 95% CI [1.326–3.131]; p=.0009; Figure 1 D).

Potential roles of *APOE4* genotypes for seroprevalence of brain-directed AB

Determination of *APOE4* genotypes in our population resulted in the expected range of around 20% (heterozygous 17.41%; homozygous 1.24%). Surprising at first view, however, regression analysis revealed that *APOE4* carriers with their known 'leaky' BBB (12, 13, 66-68) have a lower chance of being AB seropositive (OR=0.766, 95% CI [0.625-0.933], p=.009). Due to effects of brain-bound AB, seropositive compared to seronegative *APOE4* carriers might have a higher prevalence or severity of neuropsychiatric phenotypes. However, evaluating just presence/absence of a disease as readouts did not yet support this idea (p>.05 for all chi-squares).

Influence of previous neurotrauma on seroprevalence of brain-directed AB

As an environmental risk factor, we evaluated neurotrauma in a dichotomous fashion, dependent on symptom severity (Table 3). Indeed, neurotrauma was associated with a higher chance of carrying serum NMDAR1-AB of the IgM class (OR=1.599; 95% CI [1.022-2.468], p=.036).

DISCUSSION

The present work has been designed to provide a thus far lacking, comprehensive investigation of brain-directed serum AB which should assist clinicians as well as basic researchers in putting AB findings in more solid perspective. We investigated seroprevalence and potential predictors of 49 selected, brain-directed AB in >7000 subjects, healthy or suffering from neuropsychiatric diseases, a number never analyzed and reported before. In fact, thousands of different AB, likely belonging to the physiological autoimmune repertoire of individual mammals, circulate in blood (7). Brain-directed AB may gain pathophysiological significance as they can substantially modulate brain function when crossing the BBB in sufficient amounts or upon their intrathecal production (1). Here, we report humoral autoimmunity against brain-antigens equally frequent across health and disease, with overall >16% carriers of one or more of these selected AB, and with age, gender, genetic predisposition and brain injury as predictors. We note that our present AB selection represents only a small part of circulating AB (7), but findings obtained with them may be widely representative.

The global age association of AB seroprevalence seemed mainly driven by NMDAR1-AB. We note, however, that grouping all non-NMDAR1-AB, due to their overall low seroprevalence, essentially generates a 'mixed bag', i.e., not all AB may follow the same rules. Nevertheless, there seems to be a general tendency of age association at least for many of these AB. Significance of gender association in turn is driven by the heterogeneous non-NMDAR1-AB 'bag' – with higher seroprevalence of males. This is somewhat unexpected, since females are more affected by autoimmune disorders (69-74). Together, the observed age and gender dependence of seroprevalence of our selected 49 anti-brain AB, their apparent lack of disease association, both general and with neuropsychiatric subgroups, and their similar overall Ig class distribution and titer ranges may represent a more general picture to be expected from thousands of serum AB (7, 8).

As NMDAR1-AB of the IgG class are often connected to autoimmune encephalitis, we screened the literature to compare the titer values found here with those of patients with confirmed anti-NMDAR encephalitis. In a recently published Dutch cohort of anti-NMDAR encephalitis patients (n=104), two sets of NMDAR-1 IgG titer ranges, also determined by commercial cell-based assays from Euroimmun, were reported: (i) Subjects <45 years with titer median 1:800 (range 1:100-1:6,400) and (ii) subjects >45 years with titer median of 1:200 (range 1:100-1:12,800). Highest median in the present work was 1:100 (overall median 1:32, ranges 1:10-1:1,000), which we expected, as no individuals in our cohort were diagnosed with anti-NMDAR encephalitis. Notably, however, out of 55 individuals, positive for NMDAR-1 IgG, 18 (32.73%) had titers of 1:100 or higher, thus were comparable to numbers presented in the Dutch study (75).

In our genetic approaches to identify potential predisposing factors for seroprevalence of AB against brain-antigens, we performed a GWAS of NMDAR1-AB carriers versus non-carriers. Even though the obtained genome-wide significant hits do not allow deeper mechanistic insight at this point (as with most GWAS studies), they underline a genetic influence at least on NMDAR1-AB carrier status. An interesting find, however, may be *TENM3,* a gene previously associated with childhood autoimmune diseases (65), which encodes a large transmembrane protein expressed in neurons, possibly involved in the regulation of neuronal development (76).

Next, we conducted a hypothesis-driven analysis of immune-checkpoint genotypes (SNPs) for *CTLA-4*, *PD-1*, or its ligand, *PD-L1,* that resulted in 5 SNPs associated with AB seroprevalence. These genes are expressed by T-cells and serve as control elements of their immune response. Immune checkpoint inhibitors block these molecules and enhance antitumor T-cell activity (77, 78). While providing clinical benefits in a percentage of patients with advanced cancers, they are usually associated with a remarkable spectrum of immune-related adverse events, including autoimmunity (79, 80). CTLA-4 for example is an important regulator of the immune response, i.e., reactivity to foreign and self-antigens. Allelic variation of *CTLA-4* or CTLA-4 blockade by anti-CTLA4 treatment influences the signaling threshold of CD4 T-cells (79, 81), thereby augmenting antitumor immunity but also exacerbating or inducing autoimmune disease. We had previously shown in a smaller sample that *CTLA4* genotypes predispose to serum NMDAR1-AB in humans (19). Accumulating 5 risk SNPs of immune-checkpoint genes revealed effects not only on humoral anti-brain autoimmunity, but also on likelihood of neurodegenerative disease. Provided replication in independent samples, this would indicate a role of the genetic immune checkpoint constellation also for neurodegeneration.

APOE4 genotypes are known to be risk factors for various diseases, e.g., Morbus Alzheimer, or to predict unfavorable outcomes, e.g., of stroke or brain injury. These risks may be related to their negative influence on BBB integrity (12, 13, 66-68). We thus wondered whether such genetically induced higher accessibility of brain tissue to the immune system would be reflected in humans by enhanced seroprevalence of AB directed against brain-antigens, as hypothesized by some authors for ischemic stroke or brain injury (82). Determination of *APOE4* genotypes in our population resulted in the expected range of around 20%. Surprising at first view, however, *APOE4* carriers have a lower chance of being AB seropositive. This phenomenon is in good agreement with our earlier findings in *ApoE* KO mice (19) and may well be explained by the chronically leaky BBB. Brain-directed AB can under these circumstances readily cross the dysfunctional BBB and specifically bind to brain tissue, which acts as 'immunoprecipitator', as demonstrated in experimental work in *ApoE* KO mice (11). This finding spontaneously suggested that seropositive compared to seronegative *APOE4* carriers might have a higher prevalence or severity of neuropsychiatric phenotypes due to the effects of bound AB. However, just appraising presence of a disease did not back this assumption. We note as important limitations, that we did not consider in this study the severity of disease symptoms, and that the immunoprecipitator role of the brain, efficiently extracting AB from the circulation, may lead to false negative seroprevalence data.

A recognized inducer of acute BBB breakdown is traumatic brain injury. In addition, multiple, mechanistically widely unexplained, late downstream sequelae of neurotrauma are known, e.g., various organ dysfunctions and increased risk of mental health problems (83-85). Some of these consequences might be autoimmune-mediated and autoimmunity in turn a corollary of BBB disruption (82). In fact, a standardized small brain lesion in mice led to BBB breakdown and months later to increased NMDAR1-AB seroprevalence (19). Here we show also in humans first exploratory signals that neurotrauma, as environmental risk factor, was associated with a higher chance of carrying serum NMDAR1-AB of the IgM class. As after ischemic stroke, this finding may indicate potential long-term consequences of ongoing presence of circulating AB, e.g. years later neuropsychiatric symptoms including cognitive dysfunction and fatigue (86).

LIMITATIONS

Despite evaluating >7000 subjects with >16% seropositive individuals among them, total N numbers can never be large enough, particularly when subgroups are to be assessed. In addition to some limitations already mentioned above, appraising a ‘mixed bag of AB’, put together to compare with the highly frequent NMDAR1-AB, has its clear confines, as not all AB may follow the same rules. But it certainly is a worthwhile start in our struggle to understand their roles and functions. In contrast, NMDAR1-AB can be analyzed separately, and GWAS even resulted in genome-wide significant hits, however, of still unknown function. Certainly, a replication GWAS would be desirable to confirm our findings. Also, detection of autoantibodies was limited here to commercially available *in vitro* diagnostic assays, as we focused on clinical relevance. However, in some cases, the use of live cell-based assays for NMDAR1-AB may be desirable which are believed to be more sensitive (87). The fact that the 49 AB are just a selection and 'represent' probably thousands of others may be seen as another limitation. Here, use of tissue-based assays can be useful for the identification of novel CNS-directed autoantibodies (88), but this was clearly not the purpose of the present study. Another limitation is that not all potential predisposing factors could be investigated here. Viral infections are among them, and also other influences, e.g., the microbiome, have been suggested, but could not be included in the present study.

CONCLUSIONS

Humoral autoimmunity against brain-antigens is frequent across health and disease, and predicted by age, gender, genetic predisposition, and brain injury. Important for clinical practice, seroprevalence, Ig class or titers alone do not predict disease. Nevertheless, serological testing of brain-directed AB is of high diagnostic and therapeutic importance once their syndrome relevance is carefully considered in full context using multimodal approaches (including cerebrospinal fluid analysis, magnetic resonance imaging and electroencephalography). Much work needs to be done to better understand the physiological significance of circulating AB and to clearly identify situations that lead to their pathological consequences.

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List of abbreviations

Apolipoprotein E ε4 allele - APOE4

Autoantibodies - AB

Blood-brain-barrier - BBB

Cytotoxic T-lymphocyte-associated protein 4 - CTLA-4

Genome-wide association study - GWAS

Göttingen Research Association for Schizophrenia - GRAS

Immunoglobulin - Ig

Neurotrauma - NT

Programmed cell death protein 1 - PD-1

Programmed cell death protein ligand 1 - PD-L1

Single nucleotide polymorphism - SNP

Declarations

Ethical approval

The GRAS data collection has been approved by the ethical committee of the Georg-August-University of Göttingen (master committee) as well as by the respective local regulatories/ethical committees of all collaborating centers.

Data availability statement

Due to the data privacy agreement, specific patient data will not be shared.

Conflict of Interest Statement

Winfried Stöcker is head and Bianca Teegen full-time employee of a diagnostic reference laboratory, integrated into patient care, collaborating with the company *Euroimmun*, nowadays *PerkinElmer*. All other authors declare no subject-related conflict of interest.

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Author Contributions

Concept, design: HE and VDG

Drafting manuscript and display items: HE, VDG and JBHW.

Data acquisition: VDG, JBHW, CoW, CaW, NB, BT, FL, WS, FL, MB, FZ, KAN, HE.

All authors read and approved the final version of the manuscript.

LEGEND to FIGURE 1

A. Predicted NMDAR1-AB seropositivity per age group and gender. Note the strong association between predicted seropositivity and age. Males are more likely to be NMDAR1-AB seropositive, although the lower limit of the CI goes slightly below 1.

B: Predicted non-NMDAR1-AB seropositivity per age group and gender. Males are more likely to be non-NMDAR-1 AB seropositive, but the association between predicted seropositivity and age is weaker compared to NMDAR1-AB. *Note that we are considering a 'mixed bag' of AB which may not all follow the same laws.*

C. Manhattan plot of genome-wide association analysis for NMDAR1-AB seropositivity. The x axis represents chromosomal position and the y axis gives the significance (–log10(P); 2-tailed) of association as calculated by PLINK's Genotypic (2df) test.

D. Chi-square comparison between dichotomously divided groups of individuals with 0-3 versus 4-5 immune-checkpoint risk genotypes in the accumulation model. Individuals with 4-5 risk genotypes are more likely to be AB seropositive and to have a disease diagnosis. Dividing the latter into classical psychiatric and neurodegenerative disease diagnoses reveals that the disease association of the accumulation model is mainly driven by neurodegeneration.

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Table 1. Characteristics of the top 15 most frequent anti-brain antigens for autoantibody seroprevalence screening

Antigen	Full name	Location	Function	Associated diseases/syndromes	First description of autoimmune association
NMDAR1	Glutamate ionotropic receptor NMDA type subunit 1	Extracellular	Obligatory subunit of tetrameric NMDA-receptors, which have a major role in excitatory neurotransmission	Anti-NMDAR-encephalitis(30), Herpes simplex encephalitis(25), Japanese encephalitis(31)	Dalmau 2007, *Ann Neurol(30)*
Amphiphysin	Amphiphysin	Intracellular/ synaptic	Presynaptic vesicle protein, involved in vesicle endocytosis	Stiff-person syndrome(32, 33), breast cancer(32), SCLC(2), (limbic) encephalomyelitis(2), neuropathy(34)	De Camilli 1993, *J Exp Med(32)*
KCNA2	Potassium voltage-gated channel subfamily A member 2	Extracellular	Voltage gated potassium channel, neuronal excitability	Neuromyotonia(35)	Hart 1997, *Ann Neurol(35)*
ARHGAP26	Rho GTPase activating protein 26	Intracellular/ somata/neuropil	Clathrin independent endocytosis	Subacute inflammatory cerebellar ataxia(36)	Jarius 2010, *J Neuroinflamm(36)*
GFAP	Glial fibrillary acidic protein	Intracellular	Part of cytoskeleton, maintenance of astrocytic structure	Autoimmune GFAP astrocytopathy(37), meningoencephalitis(34), Alzheimer's disease(38), traumatic brain injury(39)	Fang 2016, *JAMA Neurol(37)*
CASPR2	Contactin-associated protein 2	Extracellular/ neuropil	Cell adhesion protein, antigen is VGKC associated protein	Neuromyotonia(2, 40), Morvan's syndrome(2, 40), neuropathic pain(2), limbic encephalitis(34, 40), cerebellitis/cerebellar degeneration(34)	Irani 2010, *Brain(40)*
MOG	Myelin oligodendrocyte glycoprotein	Extracellular/ outer myelin sheet	Cell adhesion molecule in oligodendrocytes, maintenance of myelin structure	Acute disseminated encephalomyelitis(2, 41), multiple sclerosis(41, 42), neuromyelitis optica spectrum disorder(41), cortical/subcortical encephalitis(34), brainstem encephalitis(34)	Xiao 1991, *J Neuroimmunol*
Homer-3	Homer protein homolog 3	Intracellular/ cytoplasm	Postsynaptic calcium responses in dendritic spines of Purkinje cells, modulating activity of metabotropic glutamate-receptors	Cerebellar ataxia(43), cerebellitis(44)	Zuliani 2007, *Neurology(43)*
KCNA1	Potassium voltage-gated channel subfamily A member 1	Extracellular	Voltage gated potassium channel, neuronal excitability	Neuromyotonia(35)	Hart 1997, *Ann Neurol(35)*
GLRA1b	Glycine receptor alpha 1 isoform b	Extracellular	Alpha1 subunit of inhibitory glycine receptor	Progressive encephalopathy with rigidity and myoclonus(45, 46), epilepsy(46)	Hutchinson 2008, *Neurology(45)*
GAD65	Glutamate decarboxylase 2	Intracellular/ cytoplasm	Intracellular / presynaptic protein involved in neurotransmitter synthesis	Stiff-person syndrome(33, 47), limbic encephalitis(34), cerebellitis/cerebellar degeneration(34), encephalomyelitis(34)	Solimena 1988, *N Eng J Med(47)*
Ma2	PNMA family member 2	Intracellular/ nuclear	Possibly involved in positive regulation of the apoptotic process	Limbic encephalitis(34, 48), brainstem encephalitis(34, 48), diencephalic encephalitis(34)	Voltz 1999, *New Eng J Med(48)*
Yo	Cerebellar degeneration related protein 2	Intracellular/ nuclear	DNA binding protein	Paraneoplastic cerebellar degeneration(49, 54), cerebellitis/cerebellar degeneration(34)	Peterson 1992, *Neurology(54)*
NF155	Neurofascin 155 kD isoform	Extracellular	Glial cell adhesion, expressed in cell bodies of oligodendrocytes	Multiple sclerosis(50, 51), chronic inflammatory demyelinating polyneuropathy(51)	Mathey 2007, *J Exp Med(50)*
AP3B2	Adaptor Related Protein Complex3Subunit Beta2	Intracellular/ Golgi apparatus	Neuron specific vesicle coat protein, controlling levels of selected membrane proteins in synaptic vesicles	Paraneoplastic cerebellar degeneration(49, 52), autoimmune cerebellar ataxia(53)	Newman 1995, *Cell(52)*

Table 2. Overview of the top 15 most frequent brain autoantibodies according to identified seroprevalence

Disorders/diseases	Schizophrenia Schizoaffective	Affective	Personality & Addiction	Neuro-developmental	Stroke	Neuro-degenerative	Autoimmune	ALL Diseases	Healthy Controls	ALL Subjects
No. Individuals†	**2043** (1818-2043)	**267** (264-267)	**334** (193-333)	**141**	**442**	**349** (310-349)	**701**	**4277** (3909-4265)	**2748** (2391-2735)	**7025** (6300-7000)
Male, %	**64.90%**	**50.56%**	**56.29%**	**65.25%**	**54.75%**	**59.89%**	**27.39%**	**55.74%**	**55.93%**	**55.81%**
Age, yr ± SD	**40.3 ± 13.1**	**47.7 ± 15.4**	**35 ± 12.9**	**29.7 ± 9.9**	**68.3 ± 12.5**	**61.6 ± 14.8**	**41.7 ± 13.7**	**44.8 ± 16.9**	**35 ± 13.1**	**41 ± 16.2**
Any AB Total No.	2043	267	334	141	442	349	701	4277	2748	7025
Seropositive No. (%)	346 (16.94)	62 (23.13)	34 (10.18)	12 (8.51)	147 (33.26)	41 (11.75)	91 (12.98)	733 (17.14)	400 (14.56)	1133 (16.13)
Seropositive, males	230	38	25	7	87	27	34	448	240	688
IgM / IgA / IgG #	134 / 148 / 141	21 / 26 / 25	7 / 20 / 13	6 / 5 / 5	69 / 58 / 49	31 / 25 / 17	39 / 39 / 47	307 / 321 / 297	155 / 166 / 180	462 / 487 / 477
NMDAR-1 Total No.	2043	264	333	141	442	341	701	4265	2735	7000
Seropositives, No. (%)	158 (7.73)	31 (11.74)	12 (3.6)	3 (2.13)	84 (19)	31 (9.09)	34 (4.85)	353 (8.28)	183 (6.69)	536 (7.66)
Seropositives, males	94	17	7	1	46	22	12	199	117	316
IgM / IgA / IgG No.	87 / 91 / 12	15 / 17 / 4	3 / 10 / 0	2 / 2 / 0	56 / 39 / 4	28 / 20 / 15	22 / 23 / 3	213 / 202 / 38	105 / 107 / 17	318 / 309 / 55
Titer range IgM	1:10-1:3200	1:10-1:320	1:32-1:320	1:10-1:100	1:10-1:1000	1:10-1:1000	1:10-1:1000	1:10-1:3200	1:10-1:1000	1:10-1:3200
Titer range IgA	1:10-1:3200	1:10-1:100	1:10-1:100	1:32-1:32	1:10-1:1000	1:10-1:1000	1:10-1:1000	1:10-1:3200	1:10-1:1000	1:10-1:3200
Titer range IgG	1:10-1:320	1:32-1:100	-	-	1:10-1:32	1:32-1:1000	1:32-1:100	1:10-1:1000	1:10-1:320	1:10-1:1000
IgM / IgA / IgG median	1:32 / 1:32 / 1:32	1:100 / 1:32 / 1:66	1:32 / 1:32 / -	1:32 / 1:32 / -	1:32 / 1:32 / 1:10	1:32 / 1:100 / 1:100	1:32 / 1:100 / 1:32	1:32 / 1:32 / 1:32	1:32 / 1:32 / 1:32	1:32 / 1:32 / 1:32
KCNA2 Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	26 (1.43)	5 (1.87)	3 (1.55)	1 (0.71)	8 (1.81)	4 (1.15)	4 (0.57)	51 (1.3)	35 (1.46)	86 (1.37)
Seropositives, males	24	4	3	1	6	2	1	41	24	65
IgM / IgA / IgG, No.	1 / 6 / 20	1 / 0 / 4	1 / 0 / 2	0 / 0 / 1	0 / 2 / 6	0 / 3 / 1	0 / 2 / 2	3 / 13 / 36	3 / 8 / 26	6 / 21 / 62
Titer range IgM	1:32	1:10	1:32	-	-	-	-	1:10-1:32	1:10-1:32	1:10-1:32
Titer range IgA	1:32-1:320	-	-	-	1:10-1:320	1:100-1:320	1:10-1:100	1:10-1:320	1:10-1:100	1:10-1:320
Titer range IgG	1:10-1:1000	1:32-1:32	1:100-1:320	1:100	1:10-1:3200	1:32	1:10-1:10	1:10-1:3200	1:10-1:1000	1:10-1:3200
IgM / IgA / IgG median	1:32 / 1:100 / 1:66	1:10 / - / 1:32	1:32 / - / 1:210	- / - / 1:100	- / 1:100 / 1:66	- / 1:320 / 1:32	- / 1:32 / 1:10	1:32 / 1:100 / 1:32	1:10 / 1:32 / 1:66	1:21 / 1:32 / 1:32
Amphiphysin Total No.	2043	264	333	141	442	310	701	4234	2726	6960
Seropositives, No. (%)	28 (1.37)	6 (2.27)	3 (0.9)	0 (0)	10 (2.26)	1 (0.32)	0 (0)	48 (1.13)	39 (1.43)	87 (1.25)
Seropositives, males	21	3	2	0	6	1	0	33	22	55
IgM / IgA / IgG, No.	1 / 7 / 23	1 / 1 / 5	2 / 3 / 3	0 / 0 / 0	0 / 6 / 6	0 / 1 / 0	0 / 0 / 0	4 / 18 / 37	4 / 14 / 29	8 / 32 / 66
Titer range IgM	1:320	1:100	1:32	-	-	-	-	1:32-1:320	1:10-1:320	1:10-1:320
Titer range IgA	1:10-1:32	1:32	1:10-1:100	-	1:10-1:100	1:32	-	1:10-1:100	1:10-1:100	1:10-1:100
Titer range IgG	1:10-1:100	1:10-1:100	1:10-1:100	-	1:10-1:32	-	-	1:10-1:100	1:10-1:100	1:10-1:100
IgM / IgA / IgG median	1:320 / 1:32 / 1:32	1:100 / 1:32 / 1:32	1:32 / 1:100 / 1:10	-	- / 1:32 / 1:21	- / 1:32 / -	-	1:100 / 1:32 / 1:32	1:100 / 1:32 / 1:32	1:100 / 1:32 / 1:32
GFAP Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	22 (1.21)	4 (1.5)	1 (0.52)	0 (0)	9 (2.04)	0 (0)	1 (0.14)	37 (0.95)	21 (0.88)	58 (0.92)
Seropositives, males	14	3	0	0	6	0	1	24	13	37
IgM / IgA / IgG, No.	5 / 7 / 16	1 / 2 / 2	0 / 0 / 1	0 / 0 / 0	1 / 2 / 8	0 / 0 / 0	0 / 0 / 1	7 / 11 / 28	1 / 2 / 20	8 / 13 / 48
Titer range IgM	1:320-1:1000	1:320	-	-	1:100	-	-	1:100-1:1000	1:100	1:100-1:1000
Titer range IgA	1:100-1:1000	1:100-1:320	-	-	1:100-1:100	-	-	1:100-1:1000	1:320-1:1000	1:100-1:1000
Titer range IgG	1:100-1:3200	1:100-1:320	1:320	-	1:100-1:1000	-	1:320	1:100-1:3200	1:100-1:1000	1:100-1:3200
IgM / IgA / IgG median	1:320 / 1:320 / 1:320	1:320 / 1:210 / 1:210	- / - / 1:320	- / - / -	1:100 / 1:100 / 1:320	- / - / -	- / - / 1:320	1:320 / 1:100 / 1:320	1:100 / 1:660 / 1:320	1:320 / 1:320 / 1:320
ARHGAP26 Total No.	2043	264	333	141	442	310	701	4234	2726	6960
Seropositives, No. (%)	16 (0.78)	6 (2.27)	3 (0.9)	0 (0)	10 (2.26)	0 (0)	0 (0)	35 (0.83)	24 (0.88)	59 (0.85)
Seropositives, males	14	4	3	0	8	0	0	29	18	47
IgM / IgA / IgG, No.	0 / 5 / 15	0 / 1 / 5	0 / 1 / 2	0 / 0 / 0	0 / 2 / 9	0 / 0 / 0	0 / 0 / 0	0 / 9 / 31	0 / 9 / 19	0 / 18 / 50
Titer range IgM	-	-	-	-	-	-	-	-	-	-
Titer range IgA	1:10-1:320	1:10	1:320	-	1:32-1:100	-	-	1:10-1:320	1:10-1:100	1:10-1:320
Titer range IgG	1:10-1:320	1:10-1:320	1:32	-	1:10-1:100	-	-	1:10-1:320	1:10-1:100	1:10-1:320
IgM / IgA / IgG median	- / 1:32 / 1:32	- / 1:10 / 1:100	- / 1:320 / 1:32	- / - / -	- / 1:66 / 1:32	- / - / -	- / - / -	- / 1:32 / 1:32	- / 1:32 / 1:32	- / 1:32 / 1:32

Table 2 – *continued.*

Disorders/diseases	Schizophrenia Schizoaffective	Affective	Personality & Addiction	Neuro-developmental	Stroke	Neuro-degenerative	Autoimmune	ALL Diseases	Healthy Controls	ALL Subjects
No. Individuals†	**2043** (1818-2043)	**267** (264-267)	**334** (193-333)	**141**	**442**	**349** (310-349)	**701**	**4277** (3909-4265)	**2748** (2391-2735)	**7025** (6300-7000)
Male, %	**64.90%**	**50.56%**	**56.29%**	**65.25%**	**54.75%**	**59.89%**	**27.39%**	**55.74%**	**55.93%**	**55.81%**
Age, yr ± SD	**40.3 ± 13.1**	**47.7 ± 15.4**	**35 ± 12.9**	**29.7 ± 9.9**	**68.3 ± 12.5**	**61.6 ± 14.8**	**41.7 ± 13.7**	**44.8 ± 16.9**	**35 ± 13.1**	**41 ± 16.2**
CASPR2 Total No.	2043	264	333	141	442	341	701	4265	2735	7000
Seropositives, No. (%)	24 (1.17)	3 (1.14)	0 (0)	2 (1.42)	2 (0.45)	0 (0)	9 (1.28)	40 (0.94)	13 (0.48)	53 (0.76)
Seropositives, males	19	1	0	0	0	0	5	25	4	29
IgM / IgA / IgG, No.	12 / 3 / 9	2 / 1 / 1	0 / 0 / 0	1 / 1 / 0	1 / 0 / 1	0 / 0 / 0	4 / 1 / 6	20 / 6 / 17	7 / 0 / 6	27 / 6 / 23
Titer range IgM	1:10-1:32	1:32	-	1:10	1:10	-	1:10-1:320	1:10-1:320	1:10-1:100	1:10-1:320
Titer range IgA	1:10-1:32	1:10	-	1:32	-	-	1:100	1:10-1:100	-	1:10-1:100
Titer range IgG	1:10-1:100	1:32	-	-	1:10	-	1:10-1:1000	1:10-1:1000	1:10-1:32	1:10-1:1000
IgM / IgA / IgG median	1:10 / 1:10 / 1:10	1:32 / 1:10 / 1:32	- / - / -	1:10 / 1:32 / -	1:10 / - / 1:10	- / - / -	1:32 / 1:100 / 1:100	1:10 / 1:32 / 1:32	1:10 / - / 1:21	1:10 / 1:32 / 1:32
MOG Total No.	2043	265	333	141	442	310	701	4234	2726	6960
Seropositives, No. (%)	17 (0.83)	0 (0)	1 (0.3)	0 (0)	8 (1.81)	3 (0.97)	1 (0.14)	30 (0.71)	11 (0.4)	41 (0.59)
Seropositives, males	9	0	1	0	3	1	1	15	8	23
IgM / IgA / IgG, No.	11 / 5 / 2	0 / 0 / 0	0 / 1 / 0	0 / 0 / 0	6 / 2 / 1	3 / 0 / 0	0 / 0 / 1	20 / 8 / 4	8 / 2 / 1	28 / 10 / 5
Titer range IgM	1:10-1:320	-	-	-	1:10-1:100	1:10-1:100	-	1:10-1:320	1:10-1:320	1:10-1:320
Titer range IgA	1:10-1:32	-	1:10-1:10	-	1:10-1:10	-	-	1:10-1:32	1:10-1:100	1:10-1:100
Titer range IgG	1:10-1:32	-	-	-	1:10	-	1:100	1:10-1:100	1:32	1:10-1:100
IgM / IgA / IgG median	1:32 / 1:10 / 1:21	- / - / -	- / 1:10 / -	- / - / -	1:32 / 1:10 / 1:10	1:32 / - / -	- / - / 1:100	1:32 / 1:10 / 1:32	1:66 / 1:32 / 1:32	1:32 / 1:10 / 1:32
Homer-3 Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	12 (0.66)	1 (0.37)	1 (0.52)	1 (0.71)	2 (0.45)	0 (0)	1 (0.14)	18 (0.46)	13 (0.54)	31 (0.49)
Seropositives, males	11	1	1	0	1	0	0	14	8	22
IgM / IgA / IgG, No.	0 / 8 / 7	0 / 0 / 1	0 / 0 / 1	0 / 1 / 0	0 / 2 / 0	0 / 0 / 0	0 / 1 / 0	0 / 12 / 9	3 / 6 / 7	3 / 18 / 16
Titer range IgM	-	-	-	-	-	-	-	-	1:100-1:320	1:100-1:320
Titer range IgA	1:100-1:1000	-	-	1:1000	1:320-1:3200	-	1:320	1:100-1:3200	1:100-1:320	1:100-1:3200
Titer range IgG	1:10-1:320	1:1000	1:100	-	-	-	-	1:10-1:1000	1:32-1:1000	1:10-1:1000
IgM / IgA / IgG median	- / 1:320 / 1:100	- / - / 1:1000	- / - / 1:100	- / 1:1000 / -	- / 1:1000 / -	- / - / -	- / 1:320 / -	- / 1:320 / 1:100	1:100 / 1:210 / 1:100	1:100 / 1:320 / 1:100
KCNA1 Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	8 (0.44)	2 (0.75)	1 (0.52)	0 (0)	4 (0.9)	0 (0)	2 (0.29)	17 (0.43)	12 (0.5)	29 (0.46)
Seropositives, males	8	2	1	0	2	0	0	13	8	21
IgM / IgA / IgG, No.	1 / 3 / 4	0 / 0 / 2	0 / 0 / 1	0 / 0 / 0	0 / 0 / 4	0 / 0 / 0	0 / 2 / 0	1 / 5 / 11	2 / 4 / 8	3 / 9 / 19
Titer range IgM	1:100	-	-	-	-	-	-	1:100	1:10-1:1000	1:10-1:1000
Titer range IgA	1:32-1:100	-	-	-	-	-	1:32-1:100	1:32-1:100	1:10-1:100	1:10-1:100
Titer range IgG	1:10-1:100	1:320-1:1000	1:320	-	1:10-1:1000	-	-	1:10-1:1000	1:10-1:320	1:10-1:1000
IgM / IgA / IgG median	1:100 / 1:32 / 1:32	- / - / 1:660	- / - / 1:320	- / - / -	- / - / 1:32	- / - / -	- / 1:66 / -	1:100 / 1:32 / 1:100	1:320 / 1:10 / 1:100	1:100 / 1:32 / 1:100
GLRA1b Total No.	2043	264	333	141	442	310	701	4234	2726	6960
Seropositives, No. (%)	5 (0.24)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (1.43)	15 (0.35)	13 (0.48)	28 (0.4)
Seropositives, males	2	0	0	0	0	0	4	6	6	12
IgM / IgA / IgG, No.	2 / 2 / 1	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0	0 / 1 / 9	2 / 3 / 10	2 / 1 / 10	4 / 4 / 20
Titer range IgM	1:32-1:32	-	-	-	-	-	-	1:32-1:32	1:10-1:32	1:10-1:32
Titer range IgA	1:10-1:32	-	-	-	-	-	1:10	1:10-1:32	1:10	1:10-1:32
Titer range IgG	1:100	-	-	-	-	-	1:10-1:100	1:10-1:100	1:10-1:320	1:10-1:320
IgM / IgA / IgG median	1:32 / 1:21 / 1:100	- / - / -	- / - / -	- / - / -	- / - / -	- / - / -	- / 1:10 / 1:32	1:32 / 1:10 / 1:32	1:21 / 1:10 / 1:32	1:32 / 1:10 / 1:32

Table 2 – *continued.*

Disorders/diseases	Schizophrenia Schizoaffective	Affective	Personality & Addiction	Neuro-developmental	Stroke	Neuro-degenerative	Autoimmune	ALL Diseases	Healthy Controls	ALL Subjects
No. Individuals†	**2043** (1818-2043)	**267** (264-267)	**334** (193-333)	**141**	**442**	**349** (310-349)	**701**	**4277** (3909-4265)	**2748** (2391-2735)	**7025** (6300-7000)
Male, %	**64.90%**	**50.56%**	**56.29%**	**65.25%**	**54.75%**	**59.89%**	**27.39%**	**55.74%**	**55.93%**	**55.81%**
Age, yr ± SD	**40.3 ± 13.1**	**47.7 ± 15.4**	**35 ± 12.9**	**29.7 ± 9.9**	**68.3 ± 12.5**	**61.6 ± 14.8**	**41.7 ± 13.7**	**44.8 ± 16.9**	**35 ± 13.1**	**41 ± 16.2**
GAD65 Total No.	2043	264	333	141	442	341	701	4265	2735	7000
Seropositives, No. (%)	9 (0.44)	1 (0.38)	1 (0.3)	0 (0)	3 (0.68)	0 (0)	3 (0.43)	17 (0.4)	9 (0.33)	26 (0.37)
Seropositives, males	8	1	1	0	2	0	0	12	7	19
IgM / IgA / IgG, No.	0 / 1 / 8	0 / 0 / 1	0 / 0 / 1	0 / 0 / 0	0 / 0 / 3	0 / 0 / 0	0 / 1 / 3	0 / 2 / 16	1 / 4 / 7	1 / 6 / 23
Titer range IgM	-	-	-	-	-	-	-	-	1:32	1:32
Titer range IgA	1:100	-	-	-	-	-	1:100	1:100-1:100	1:32-1:100	1:32-1:100
Titer range IgG	1:10-1:100	1:320	1:320	-	1:32-1:100	-	1:100-1:3200	1:10-1:3200 (1:32)	1:10-1:320	1:10-1:3200
IgM / IgA / IgG median	- / 1:100 / 1:32	- / - / 1:320	- / - / 1:320	- / - / -	- / - / 1:32	- / - / -	- / 1:100 / 1:1000	- / 1:100 / 1:32	1:32 / 1:100 / 1:32	1:32 / 1:100 / 1:32
Ma2 Total No.	2043	264	333	141	442	310	701	4234	2726	6960
Seropositives, No. (%)	7 (0.34)	1 (0.38)	4 (1.2)	0 (0)	2 (0.45)	0 (0)	0 (0)	14 (0.33)	8 (0.29)	22 (0.32)
Seropositives, males	4	1	3	0	1	0	0	9	2	11
IgM / IgA / IgG, No.	3 / 2 / 2	0 / 1 / 0	0 / 4 / 0	0 / 0 / 0	1 / 0 / 1	0 / 0 / 0	0 / 0 / 0	4 / 7 / 3	3 / 2 / 4	7 / 9 / 7
Titer range IgM	1:10-1:320	-	-	-	1:32	-	-	1:10-1:320	1:32-1:100	1:10-1:320
Titer range IgA	1:10-1:32	1:32	1:10-1:32	-	-	-	-	1:10-1:32	1:32-1:320	1:10-1:320
Titer range IgG	1:32-1:32	-	-	-	1:10	-	-	1:10-1:32	1:10-1:100	1:10-1:100
IgM / IgA / IgG median	1:320 / 1:21 / 1:32	- / 1:32 / -	- / 1:32 / -	- / - / -	1:32 / - / 1:10	- / - / -	- / - / -	1:100 / 1:32 / 1:32	1:32 / 1:100 / 1:100	1:32 / 1:32 / 1:32
Yo Total No.	2043	264	333	141	442	310	701	4234	2725	6959
Seropositives, No. (%)	5 (0.24)	1 (0.38)	0 (0)	1 (0.71)	1 (0.23)	1 (0.32)	1 (0.14)	10 (0.24)	10 (0.37)	20 (0.29)
Seropositives, males	4	1	0	1	1	1	0	8	6	14
IgM / IgA / IgG, No.	0 / 2 / 3	0 / 1 / 0	0 / 0 / 0	0 / 0 / 1	0 / 0 / 1	0 / 1 / 0	0 / 0 / 1	0 / 4 / 6	1 / 4 / 7	1 / 8 / 13
Titer range IgM	-	-	-	-	-	-	-	-	1:10	1:10
Titer range IgA	1:10-1:100	1:10	-	-	-	1:32-1:32	-	1:10-1:100	1:10-1:32	1:10-1:100
Titer range IgG	1:10-1:100	-	-	1:100	1:32	-	1:10000	1:10-1:10000	1:10-1:100	1:10-1:10000
IgM / IgA / IgG median	- / 1:32 / 1:10	- / 1:10 / -	- / - / -	- / - / 1:100	- / - / 1:32	- / 1:32 / -	- / - / 1:10000	- / 1:32 / 1:66	1:10 / 1:21 / 1:10	1:10 / 1:32 / 1:32
NF155 Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	5 (0.28)	0 (0)	0 (0)	0 (0)	3 (0.68)	0 (0)	6 (0.86)	14 (0.36)	3 (0.13)	17 (0.27)
Seropositives, males	3	0	0	0	2	0	1	6	1	7
IgM / IgA / IgG, No.	2 / 0 / 3	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0	2 / 0 / 1	0 / 0 / 0	5 / 1 / 2	9 / 1 / 6	2 / 0 / 1	11 / 1 / 7
Titer range IgM	1:10-1:10	-	-	-	1:10-1:32 (1:21)	-	1:32-1:32	1:10-1:32	1:10-1:32 (1:21)	1:10-1:32
Titer range IgA	-	-	-	-	-	-	1:100 (1:100)	1:100 (1:100)	-	1:100 (1:100)
Titer range IgG	1:10-1:100	-	-	-	1:10	-	1:10-1:32 (1:21)	1:10-1:100	1:32	1:10-1:100
IgM / IgA / IgG median	1:10 / - / 1:32	- / - / -	- / - / -	- / - / -	1:21 / - / 1:10	- / - / -	1:32 / 1:100 / 1:21	1:32 / 1:100 / 1:32	1:21 / - / 1:32	1:32 / 1:100 / 1:32
AP3B2 Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	9 (0.5)	2 (0.75)	0 (0)	0 (0)	3 (0.68)	0 (0)	1 (0.14)	15 (0.38)	2 (0.08)	17 (0.27)
Seropositives, males	5	2	0	0	2	0	1	10	2	12
IgM / IgA / IgG, No.	1 / 6 / 3	0 / 2 / 0	0 / 0 / 0	0 / 0 / 0	0 / 3 / 0	0 / 0 / 0	0 / 1 / 1	1 / 12 / 4	0 / 1 / 1	1 / 13 / 5
Titer range IgM	1:32	-	-	-	-	-	-	1:32	-	1:32
Titer range IgA	1:10-1:100	1:10-1:100	-	-	1:10-1:100	-	1:10	1:10-1:100	1:10	1:10-1:100
Titer range IgG	1:10-1:320	-	-	-	-	-	1:100	1:10-1:320	1:32	1:10-1:320
IgM / IgA / IgG median	1:32 / 1:32 / 1:32	- / 1:32 / -	- / - / -	- / - / -	- / 1:32 / -	- / - / -	- / 1:10 / 1:100	1:32 / 1:32 / 1:66	- / 1:10 / 1:32	1:32 / 1:32 / 1:32

†Range accounts for missing determinations; Ig class numbers do not always add up to the total number of seropositives, due to double and triple positives; No. = number; yr = years; SD = standard deviation; Ig = immunoglobulin.

Table 3: Severity Categories for Neurotrauma (NT)

Category	*Conditions*	*Initial rank*	*Additional NT(s)*
Mild	Head bump, nausea, laceration, or unconsciousness for <15s	1	+ 0.5
Moderate	Hematoma, hospitalization, or unconsciousness between 15s - 1h	2	+ 2
Severe	Concussion, coma, fracture, bleeding/ edema, or unconsciousness for >1h	3	+ 3

Table 4. SNPs in checkpoint inhibitor genes predispose to AB seropositivity

SNP	Allele	AAB Positive N	AAB Positive %	AAB Negative N	AAB Negative %	P	Genotype	AAB Positive N	AAB Positive %	AAB Negative N	AAB Negative %	P	Gene	Seropositivity
rs3087243	A	283	52.21%	4604	45.08%	**0.010**	AA	80	29.52%	1056	20.67%	**0.016**	**CTLA-4**	**Extracellular IgA**
	G	259	47.79%	5608	54.92%		AG	123	45.39%	2495	48.83%			
							GG	68	25.09%	1558	30.50%			
rs11571316	A	260	48.33%	4228	41.65%	**0.020**	AA	66	24.53%	889	17.51%	0.058	**CTLA-4**	**Extracellular IgA**
	G	278	51.67%	5924	58.35%		AG	128	47.59%	2450	48.27%			
							GG	75	27.88%	1737	34.22%			
rs2868042O	T	163	36.88%	3046	30.15%	**0.024**	TT	33	14.93%	494	9.78%	0.095	**PD-1**	**IgM NMDAR-1**
	C	279	63.12%	7056	69.85%		TC	97	43.89%	2058	40.75%			
							CC	91	41.18%	2499	49.47%			
rs2297137	A	31	34.44%	2381	22.39%	0.058	AA	9	20.00%	269	5.06%	**0.0004**	**PD-L1**	**IgG NMDAR-1**
	G	59	65.56%	8251	77.61%		AG	13	28.89%	1843	34.68%			
							GG	23	51.11%	3202	60.26%			
rs2297136	G	30	33.33%	5246	49.34%	**0.022**	GG	6	13.33%	1311	24.66%	**0.049**	**PD-L1**	**IgG NMDAR-1**
	A	60	66.67%	5386	50.66%		AG	18	40.00%	2624	49.36%			
							AA	21	46.67%	1381	25.98%			

- A selection of 49 anti-brain autoantibodies was analyzed in >7000 subjects

- Brain-directed autoantibodies are frequent across health and disease
- Neither seroprevalence nor immunoglobulin class nor titers alone predict disease
- Age, gender, genetic factors, and brain injury emerge as predictors of serum AB